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Final Technical Report

Total Body Calcium Analysis Using
The $^{40}Ca(n,\alpha)\ ^{37}$Reaction

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Final Technical Report

Total Body Calcium Analysis Using The $^{40}Ca(n,\alpha)\ ^{37}Ar$ Reaction

Contract NAS9-13029 - 10/01/76 to 12/01/77

Submitted to: National Aeronautics and Space Administration
Johnson Manned Space Flight Center
Houston, Texas

Submitted by: Division of Nuclear Medicine
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1.0 Introduction

1.1 Purpose of Contract

The primary purpose of this contract is to develop a low dose neutron activation technique to measure total body calcium (TBC) in-vivo. The effort has included development of irradiation and processing facilities and conduction of human studies to determine the accuracy and precision of measurement attainable with the systems.

The primary motivation for the development of the technique was to provide a means to serially quantitate charges in total body calcium during long-term zero-g conditions and simultations of zero-g conditions (i.e. bed-rest studies). The technique would also provide needed information in the study and management of metabolic bone disease such as osteoporosis.

1.2 Basis of Technique

TBC systems using total body neutron irradiation had already been developed at the time this contract was initiated. However, these systems, all based on slow neutron capture by ^{48}Ca to form ^{49}Ca, require a patient dose of 100-200 mrads (1-4). H.E. Palmer noted that ^{40}Ca was about 500 times more abundant and thus if a suitable reaction involving ^{40}Ca could be found, TBC measurements at significant lower doses might be possible.

The fast (n,α) reaction on ^{40}Ca to produce the radioactive gas ^{37}Ar, which in turn would be excreted through the lungs, became the reaction of interest. During in-vivo neutron irradiation, ^{37}Ar can only be produced from ^{40}Ca. Argon-37 decays by Auger electron emission (2.62 KeV) with a half-life of 35 days (Figure 1). The only other isotope of argon produced is ^{41}Ar which is produced in less quantity than the ^{37}Ar and is prevented from causing any interference during counting due to the short 1.8 hour half-life (5) (the ^{41}Ar decays away prior to counting the sample).

PRODUCTION AND DECAY OF RADIOACTIVE ARGON

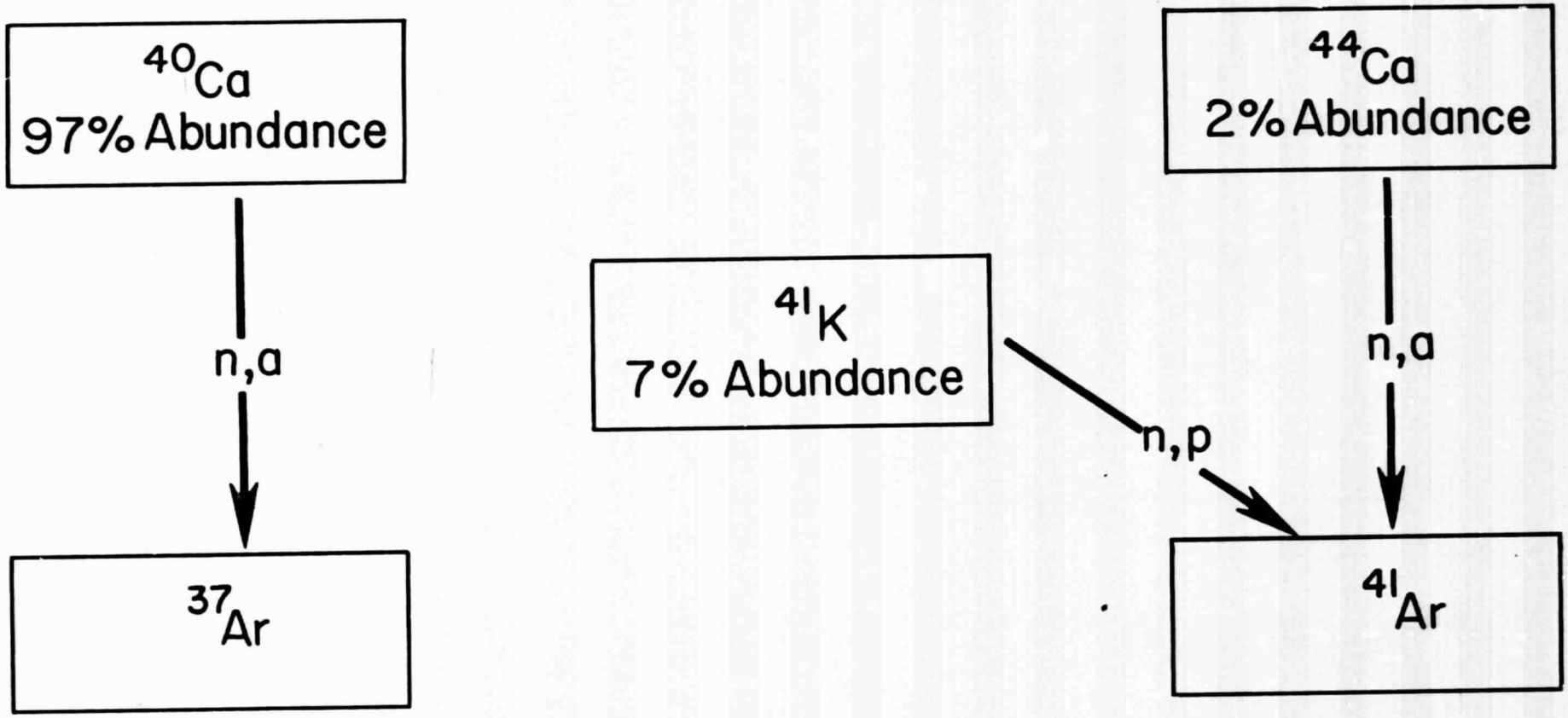


Figure 1

$t_{1/2}$ = 35.1 Days

Electron Capture Decay Emits
2.6 KeV X-Rays and Auger Electrons

$t_{1/2}$ = 1.83 Hours

Beta Decay
Emits 1.29 MeV Gamm

H.E. Palmer performed animal studies to determine the basic feasibility of a ^{37}Ar TBC system. In rats, TBC was measured with an absolute accuracy of $\pm$ 3% (6). These data also suggested that measurements of total body calcium in humans could be obtained with a neutron exposure of 10 mrad or less. These observations lead directly to the effort summarized in this report.

1.3 System Overview

The technique is based on collecting ^{37}Ar exhaled in the breath following a uniform total body irradiation by 14 MeV neutrons. The ^{37}Ar in the exhaled breath, which is directly proportional to TBC, is extracted and the radioactivity is quantitated in a low-background proportional detector system. The systems developed for human studies at the University of Washington are, for the most part, based on the systems originally described for animal studies by H.E. Palmer (6).

The ^{37}Ar technique involves the use of four systems: 1) the irradiation facility consisting of the 14 MeV neutron generator and the patient enclosure; 2) the gas collection system consisting of a closed-circuit breathing system and sampling cylinders; 3) the gas purification system which isolates the argon in the breath sample using selective absorption; and 4) the argon-37 counting system consisting of low-background proportional detectors with appropriate shielding and counting electronics.

As discussed in other sections of this report, the systems currently in operation provide an activation uniformity of $\pm$ 2.7% and an accuracy of measurement of at least $\pm$ 5.5% and a precision of measurement of $\pm$ 2.7% with a total patient dose of 10 mrads.

2.0 Activation Facility

2.1 Description of Facility

The basic facility is diagrammed in Figure 2, and consists of a 14 MeV neutron generator, a patient enclosure, dose monitors, a dose control system interfaced to the neutron generator control system, and necessary shielding.

The neutron generator is a Kaman Model A-711 producing ≈5 x 10^{10} neutrons/second into 4 Π steroidions. The generator head is enclosed in a "pillbox" made up of high density concrete blocks (7). The exit part of the "pillbox" is equipped with a collimator (Fig. 2) made from borated water-extended resin. The collimator limits the area of the direct beam to a rectangle approximately 300 cm high and 250 cm wide at the patient position.

To provide optimal uniformity of ^{40}Ca activation to all parts of the body and to allow for different body sizes, the patient enclosure was designed such that the patient is surrounded with water, keeping the total volume of the enclosure constant. A rectangular tank, 60.9 cm x 30.5 cm x 198 cm was made from 1/4" thick aluminum plate (Fig. 3). An adjustable lucite platform inside the tank allows the patient's shoulders to be positioned just below the top of the tank. The tank is mounted on top of a turntable to allow bilateral irradiation.

The radiation dose delivered to the patient is monitored during irradiation by two devices, a tissue-equivalent ion chamber and a LiI scintillator. The ion chamber, an 80 cc EG&G device used with a constant 5 ml/min. flow of tissue equivalent gas is used to turn off the neutron generator at a preset value (7). The dosimeters were calibrated against similar dosimeters used for neutron irradiation at the University of Washington cyclotron facility (8). Measurements with a graphite ion chamber indicate that the neutrons account for more than 90% of the 10 mrad patient dose.

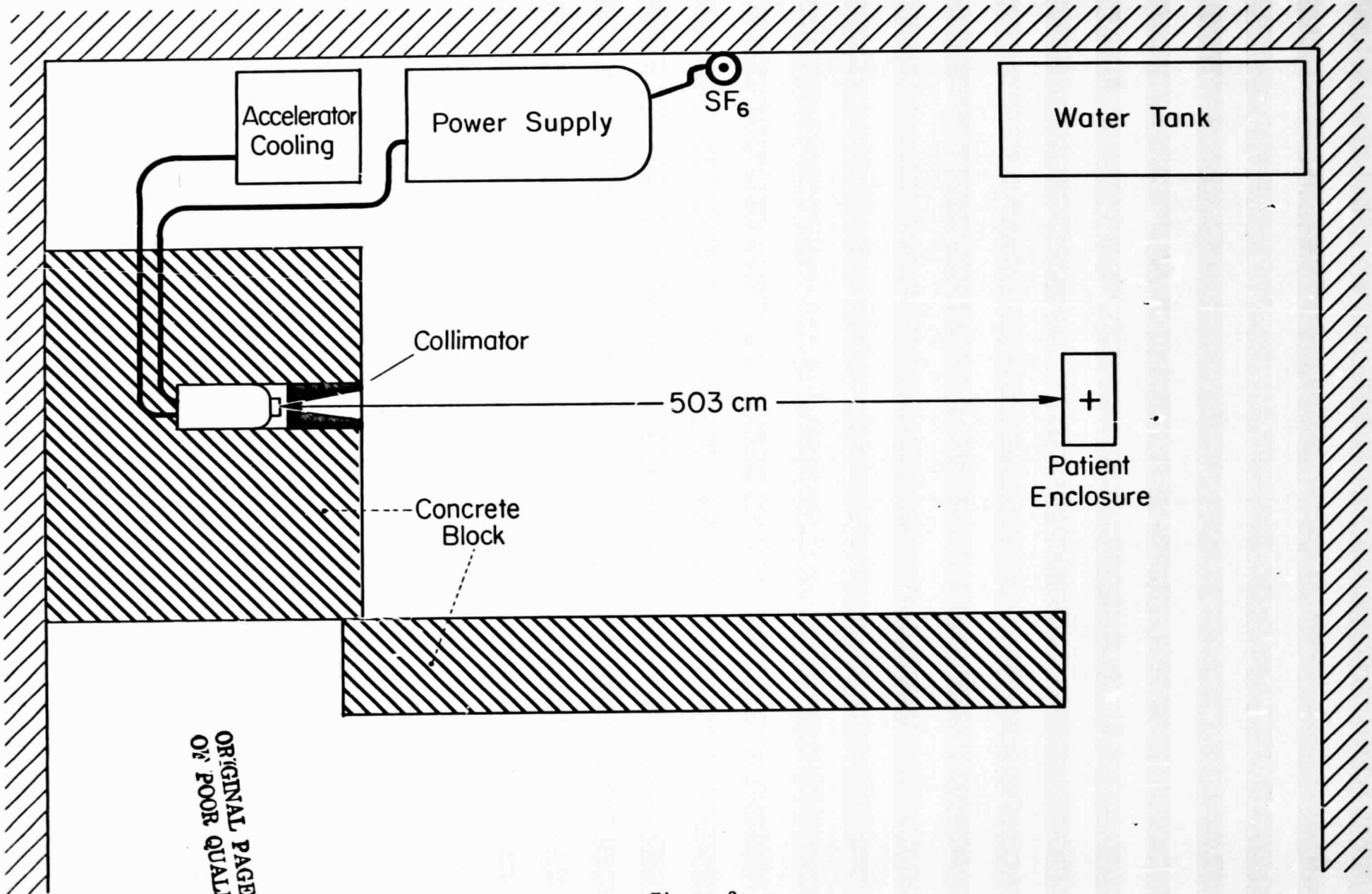


Figure 2

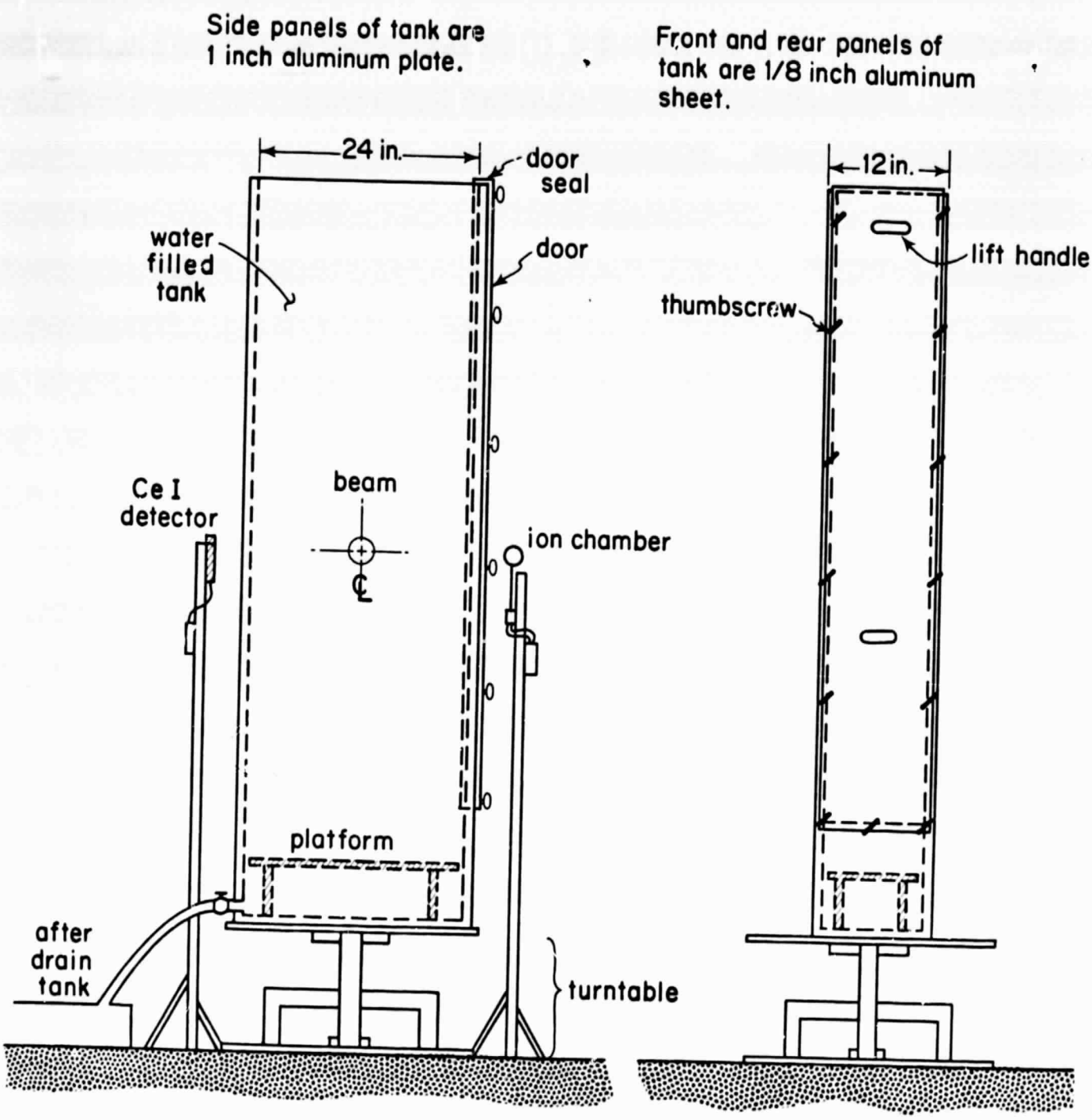

Side panels of tank are 1 inch aluminum plate.
Front and rear panels of tank are 1/8 inch aluminum sheet.
24 in.
12 in.
door seal
door
water filled tank
lift handle
thumbscrew
beam
Ce I detector
ion chamber
platform
after drain tank
turntable

Figure 3

2.2 Activation Uniformity

The uniformity of the neutron field in air was measured at the surface of the patient irradiation position by irradiating a series of aluminum pellets, 0.64 cm in diameter and 1.27 cm long, suspended in a grid pattern. The relative fast neutron flux was measured using the ^{27}Al (n,α) ^{24}Na reaction. The results are shown in Figure 4. Except for positions within 50 cm of the concrete floor, the flux distribution varied only by ± 2% over an area 203 cm x 203 cm, consistent with the expected $1/r^2$ distribution (5).

The activation uniformity inside the water filled tank was measured by irradiating vials filled with calcium nitrate solutions and quantitating the ^{37}Ar produced. The 10 ml borisilicate glass vials were filled with a solution of 100 g of $Ca(NO_3)\cdot 4H_2O$ per 100 ml water. Forty-five vials were positioned on three planes within the enclosure, such that both the center and sides of each plane were sampled at several depths. The vials were irradiated simultaneously (Fig. 5). Each vial was then removed and placed inside a gas tight container consisting of a 12 inch long, 1 inch I.D. tygon tube fitted with lucite end plates and containing 30 ml of water. The vial was broken and the ^{37}Ar removed by bubbling helium through the container and routing the gas through the argon purification system described below. The ^{37}Ar from each vial was then quantitated in a low-background proportional detector.

The results, as well as similar data obtained independently by Palmer (Palmer, Private Communication), are shown in Figure 6. For a bilateral irradiation, the uniformity of activation inside the water-filled enclosure was ± 2.7%, providing a mean yield of 1.21 pCi of ^{37}Ar/g calcium per mrad dose.

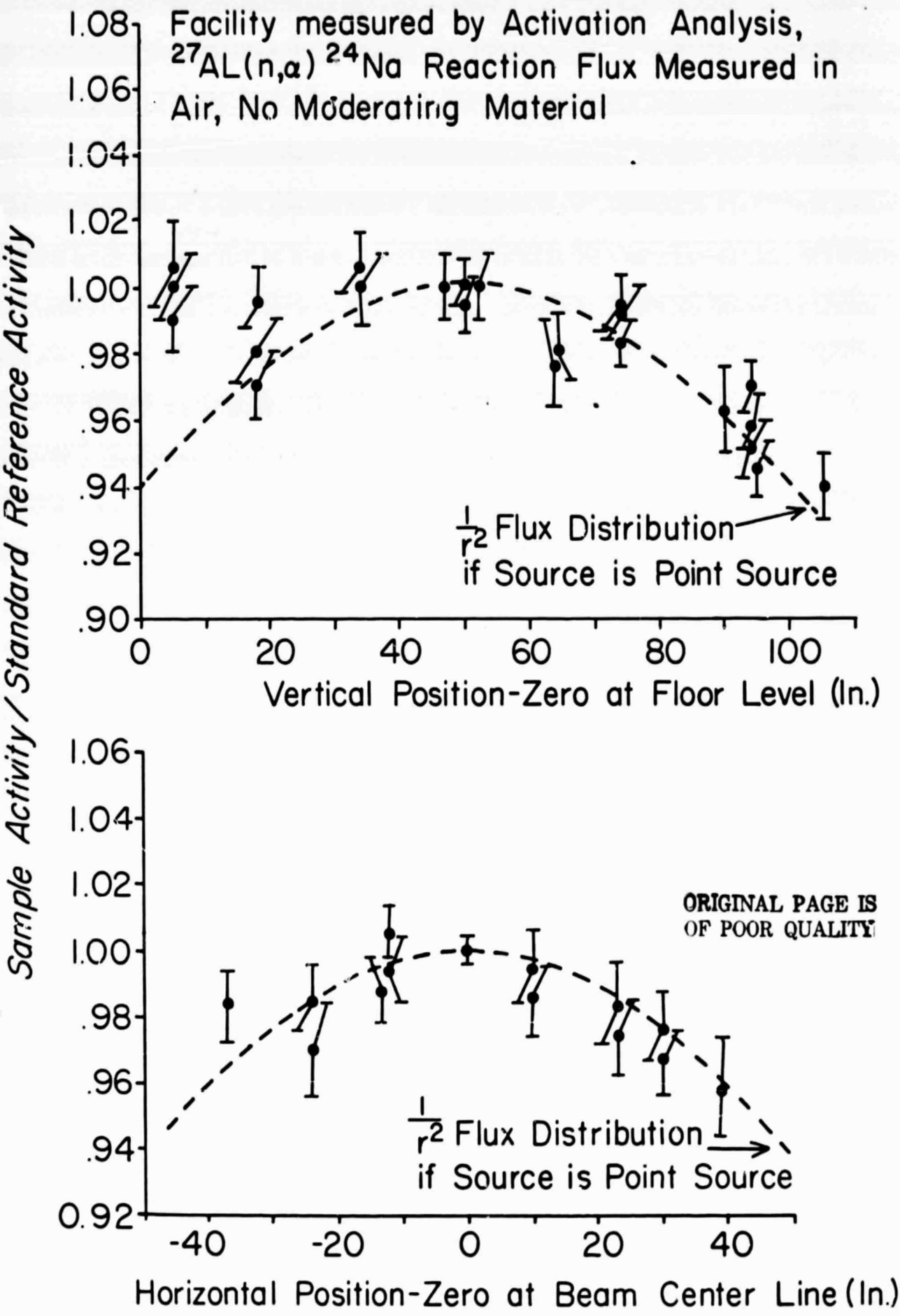


Figure 4

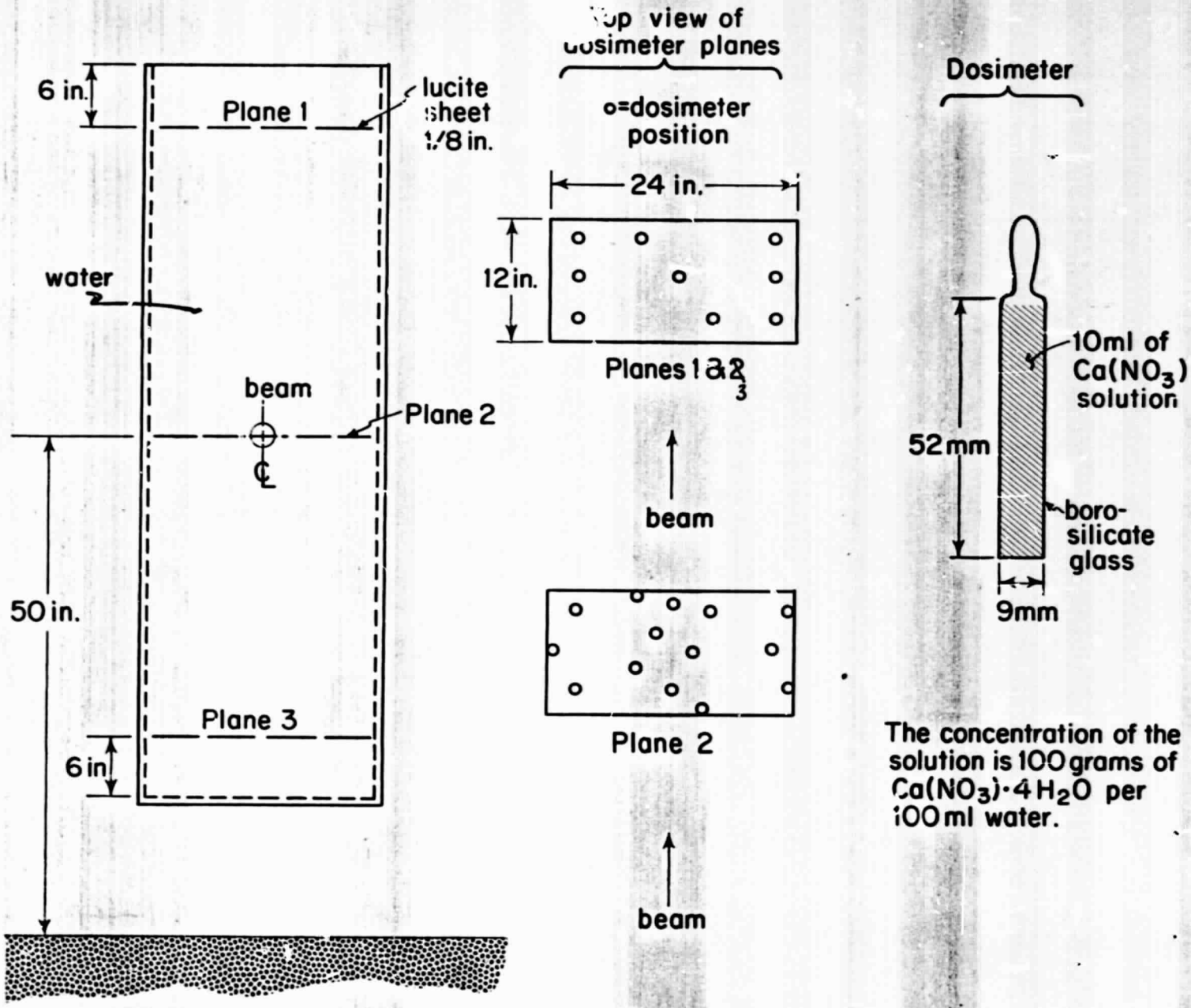


Figure 5 - Dosimeter position for patient irradiation facility uniformity.

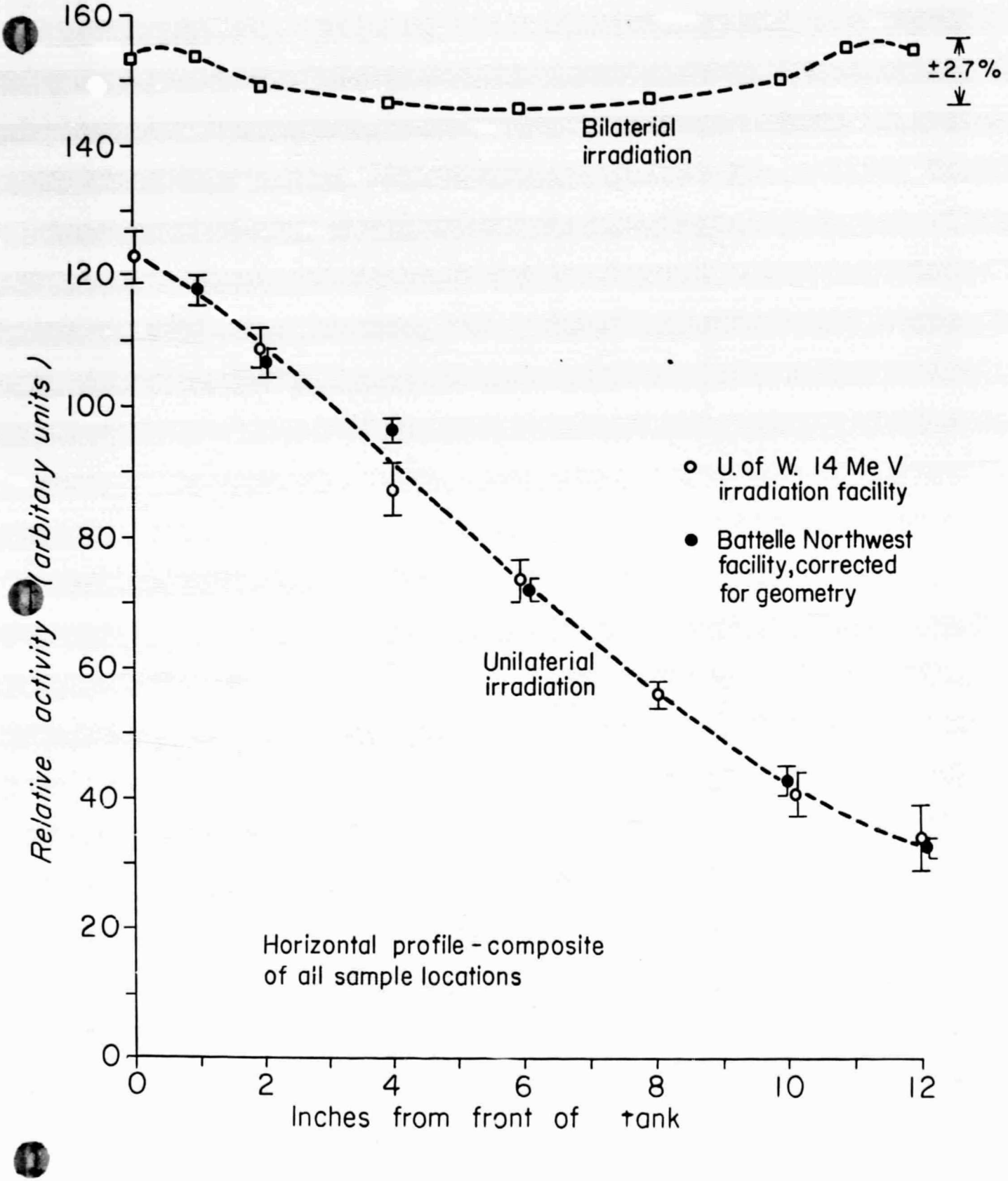

160
140
120
100
80
60
40
20
0
Relative activity (arbitary units)
±2.7%
Bilaterial irradiation
U. of W. 14 Me V irradiation facility
Battelle Northwest facility, corrected for geometry
Unilaterial irradiation
Horizontal profile - composite of all sample locations
0
2
4
6
8
10
12
Inches from front of tank

Figure 6

2.3 Discussion

For TBC studies, the patient is positioned in the tank, the door is sealed, and the tank is filled with lukewarm water. The face mask of the gas collection system is installed and then the bilateral irradiation is carried out (the turntable is rotated manually between the irradiations). The water is then removed using the large drain valve at the bottom of the tank, the patient dries off and dresses (wearing the face mask at all times) and is transported to a waiting room for the remainder of the breath collection period. The typical irradiation time, including the rotation of the enclosure, is four minutes.

The uniformity of activation for the ^{37}Ar TBC system is excellent, significantly better than the uniformity attainable in ^{49}Ca studies (7). The activation uniformity measured in our laboratories is generally better than has been reported for ^{37}Ar studies at other laboratories using neutron beams from cyclotrons (9). However, since neutron beams produced from cyclotrons have a fairly wide energy spread width, a large portion of the neutron energies are near the rapid decrease in the (n,α) reaction cross section (Figure 7), a greater fall-off of activation with depth for cyclotron based irradiation facilities is to be expected.

For final data analysis, a precise comparator standard must be irradiated with the patient. This standard corrects all measurements for any differences in neutron fluence. The standard consists of a set of four aluminum disks, each 22.9 cm in diameter and 0.64 cm thick, positioned at the corners of a rectangle, 91 cm x 198 cm, centered around the patient enclosure. After each patient irradiation, the ^{24}Na produced in the disks by the ^{27}Ar (n,α) reaction is quantitated with the use of two opposing 9"

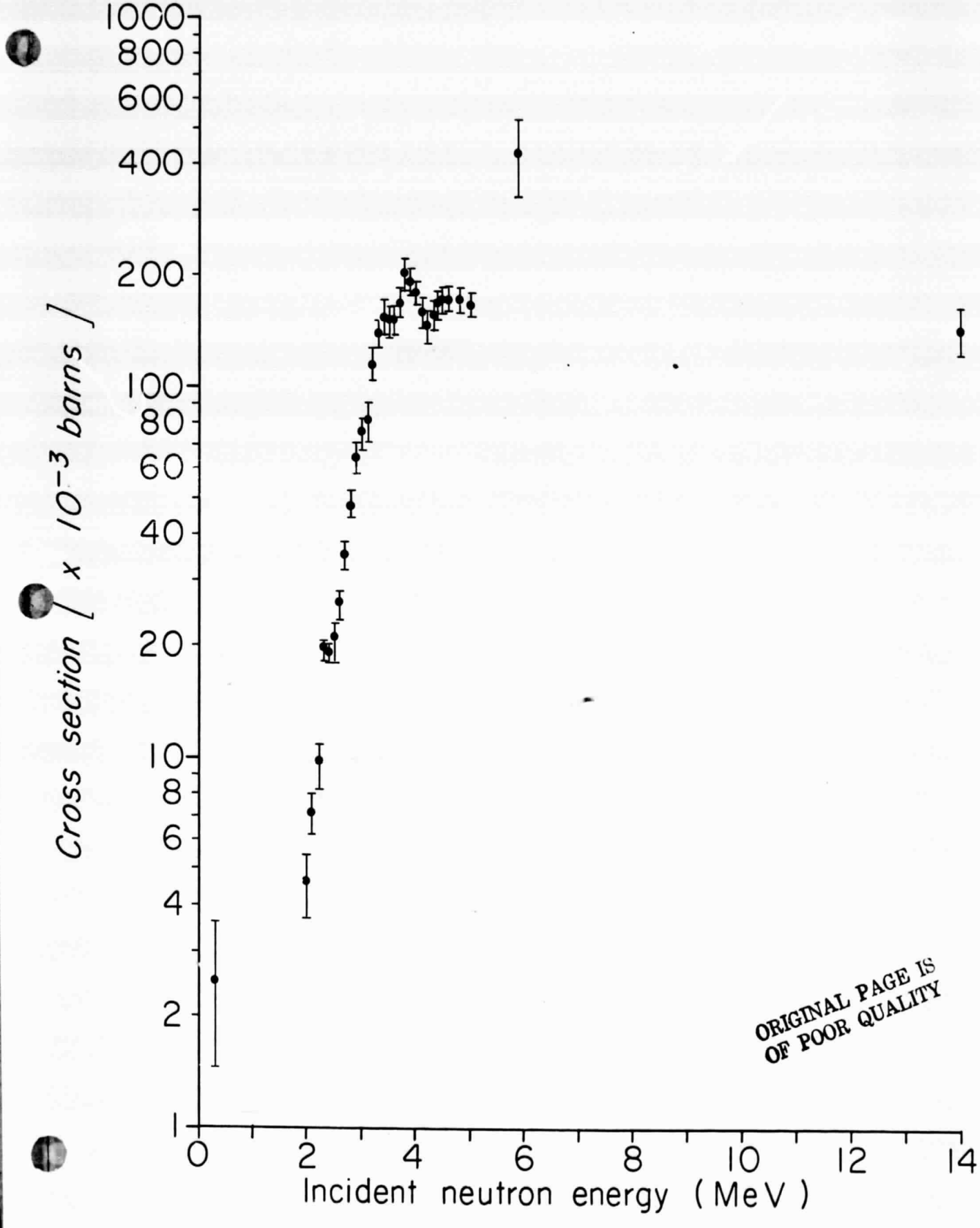


Figure 7

diameter x 4" thick NaI(Tl) detectors. Typically 10^5 counts from ^{24}Na are obtained during a 1000 second counting period at four hours post irradiation.

3.0 Gas Collection System

3.1 Description of System

During the early feasibility studies, several breath collection systems were studied. The systems all had two basic features. First, to reduce nitrogen in the breath and hence reduce processing time in the purification system, the patient is maintained on a 25% O_2 - 75% He atmosphere for five minutes prior to and during breath collection. Second, the ^{37}Ar, as well as other gases, are trapped on Type 5A molecular sieve material cooled by liquid nitrogen at the end of the breath collection period.

In early systems, the O_2-He was continuously fed to the system and the patient's breath was collected without any recirculation. Because of the large volumes, these systems could hold breath samples of only 10-15 minutes. After the first ^{37}Ar excretion rate studies, it became desirable to collect samples of 1-3 hours in length and a closed-circuit system was designed (Fig. 8). The exhaled breath is recirculated, Co_2 is removed and O_2 is replaced as needed. Thus, the total volume of gas collected in each sample is a constant, independent of the amount of time the system is in use. The system includes an expansion bellows, the position of which is monitored by two reed relays which are used to open and close a solenoid valve to the oxygen supply, a circulating fan to reduce back pressure and keep a constant flow of air moving across the face plate of the mask, a soda lime cannister to absorb CO_2, bacterial filters and valves to allow the system to be flushed with O_2-He or put on closed-circuit operation. The rebreather is installed underneath a reclining wheel-chair and the power is supplied by a 12-volt battery and charger system, making the entire system self-contained and mobile.

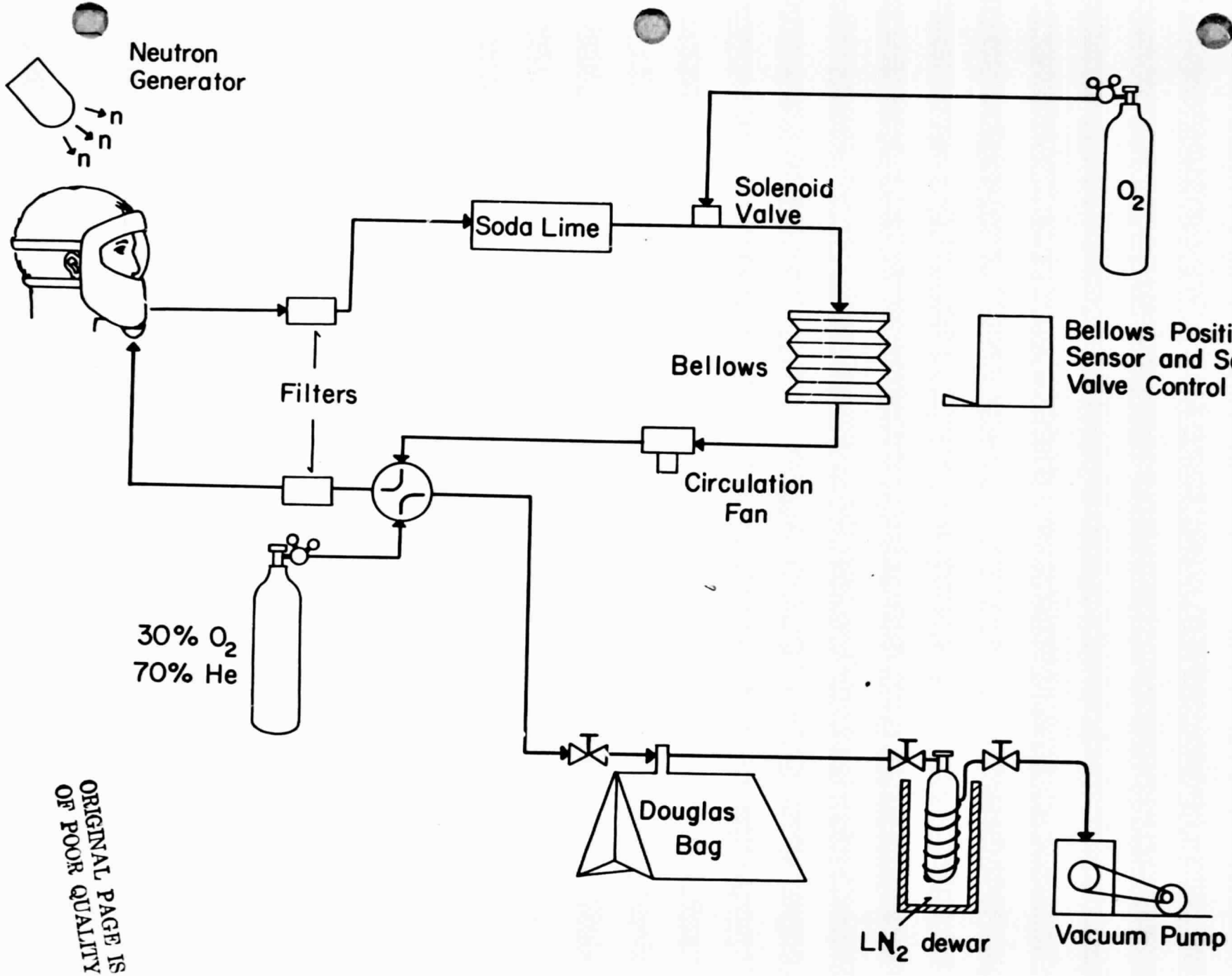


Figure 8

GAS COLLECTION SYSTEM

After the desired breath collection period has ended, the system is flushed with O_2-He and the exhaust is routed into a 100 liter Douglas bag. The gas in the Douglas bag is pumped through a 4 liter stainless steel sampling cylinder and a coil of copper tubing (Fig. 8) both of which are filled with Type 5A molecular sieve material. Since the cylinder is cooled by liquid nitrogen (LN_2), all gases except helium are trapped and the helium is pumped off, after which the cylinder can be closed off and allowed to warm up for storage prior to the gas sample being processed. The majority of oxygen, water-vapor, and CO_2 are trapped in the cylinder where the cross sectional area is sufficiently large to prevent "ice" blockage while the argon and any nitrogen are primarily trapped in the copper tubing.

The final gas sample volume to be processed is approximately 30 liters, regardless of the length of time used in collecting it.

3.2 Discussion

The system has proven to be quite reliable and well-tolerated by patients for up to six hours of continuous operation. The only long-term maintenance required, besides renewing the soda lime and gas supplies, has been periodic replacement of the bellows at about four-month intervals.

The use of a closed-circuit breathing system does raise the question of reabsorption of previously exhaled ^{37}Ar into body tissues. Since the solubility constants and diffusion rate constants for argon and nitrogen are similar (10), we used data on nitrogen absorption during the design study for the rebreathing system. Investigations of the rate of excretion from body tissues when the atmosphere the patient is breathing is suddenly shifted from normal to one with essentially no nitrogen content have been reported (11). The usual result is that 80-90% of the absorbed nitrogen is released in three hours.

Based on the nitrogen data, our assumption was that by using ultra-pure gases (argon content < 0.01%) and a reasonably large volume in the rebreathing system that the stable argon absorbed in the body (normally at equilibrium with about 1% argon concentration in the atmosphere) will be rapidly excreted from the body, minimizing reabsorption problems.

Three patients being studied for excretion rate measurements (12) were irradiated twice over a period of about six months with breath samples collected with an open circuit system on one irradiation and a closed-ciruit system on the other. The patients selected did not have a significant change of body weight between measurements. The excretion rates measured were the same (within a range of $\pm$ 5%) at each point in time for each patient. These results suggest that there was no significant reabsorption of ^{37}Ar in the closed-circuit system when it is loaded with essentially argon-free gas.

4.0 Gas Purification System

4.1 Description of System

The purification system, a simplified diagram of which is shown in Figure 9, is based on absorption of undesired gaseous products. Copper columns maintained at 400°C and previously reduced by hydrogen absorb oxygen, columns of soda lime, $CaSO_4$ and Aquasorb remove CO_2 and water vapor, after which remaining gases are first trapped on Type 5A molecular sieve material while residual helium is pumped off and then placed into a tube containing calcium at 600°C to remove nitrogen. The last step is to transfer the sample into a 30 cc proportional detector by means of a calibrated Toepler pump. The volume of gas transferred is measured and then, using calibrated volumes, sufficient stable argon and CH_4 are transferred to bring the proportional counter gas to a mixture of 90% Ar and 10% CH_4 at 1 atms. absolute.

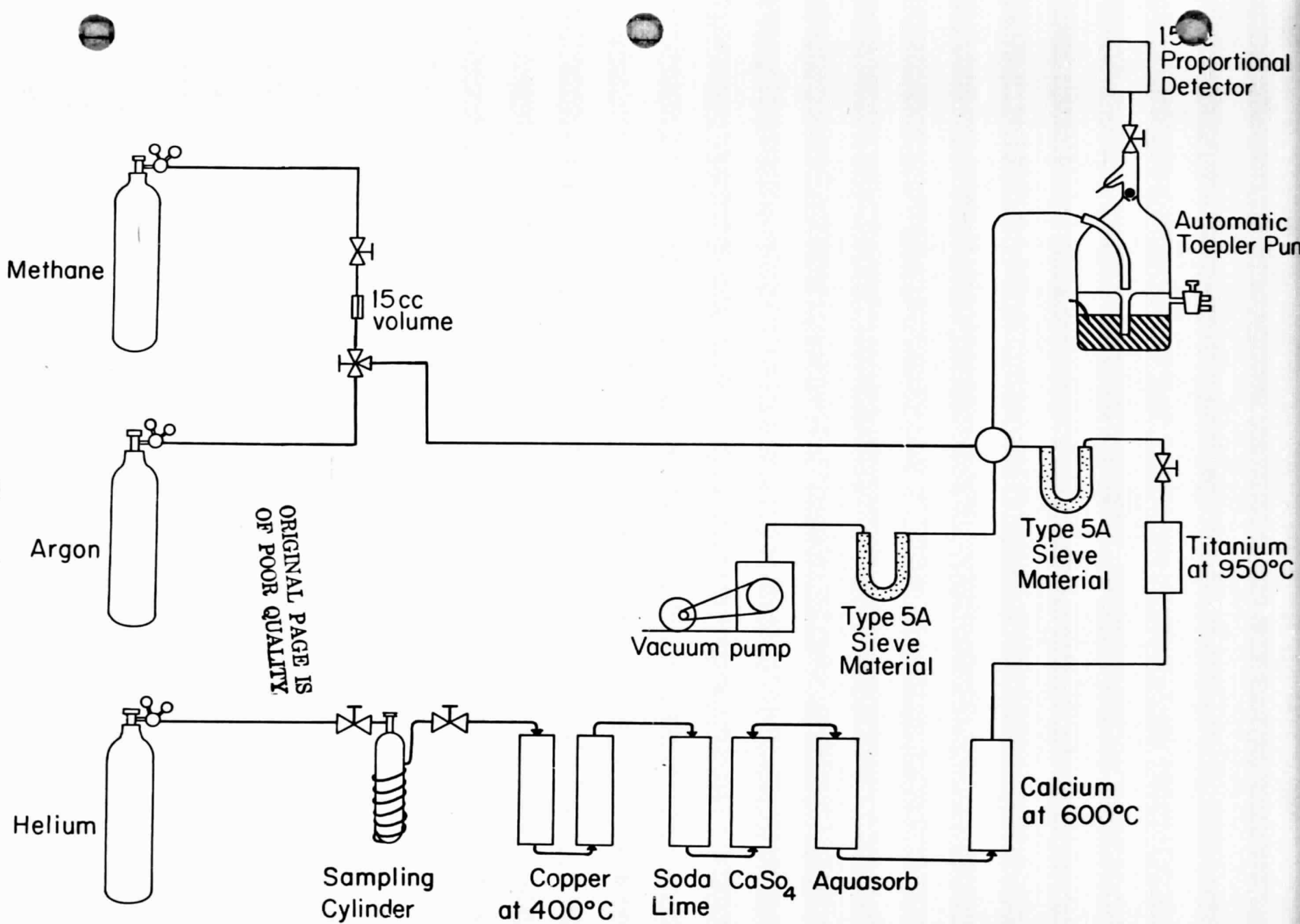


Figure 9

ORIGINAL PAGE IS OF POOR QUALITY

GAS PURIFICATION SYSTEM

4.2 Discussion

The entire purification procedure, including regeneration of the copper columns, takes approximately five hours, although only intermittent attention by a technician is required. The calcium furnace containing 550 g of calcium turnings, is sufficient for about 400 procedures before replenishment and is the only long-term maintenance item besides normal vacuum system upkeep and periodic replacement of the soda lime and $CaSO_4$ cannisters.

The system being completely manually operated does require great care in use to avoid inadvertent venting or contamination of the sample. As indicated in a more detailed schematic of the system (Fig. 10), the technician must exercise considerable care in operation. Future implementations of the ^{37}Ar technique would benefit greatly from fully automating the gas processing system.

5.0 ^{37}Ar Counting System

5.1 Description of System

The proportional detector is placed between two 9" x 4" NaI(Tl) detectors which act as cosmic ray anti-coincidence shields and the entire detector assembly is surrounded by four inches of lead (Fig. 11). The only unusual feature of the electronics is the inclusion of a pulse shape analyzer (Ortec Model 458) which is used to discriminate against noise based on the rise time of the pulses. A typical ^{37}Ar spectrum is shown in Figure 12 along with a spectrum of ^{55}Fe which is used to set the amplifier gain.

Two basic designs are used for the proportional detectors (Fig. 13). For the standard ^{37}Ar study (patient dose - 10 mrad) a 30 cc detector made out of oxygen free copper tubing is used. The detectors have an inside diameter of 15 mm with a wall thickness of 1.0 mm, Kovar feed troughs at the

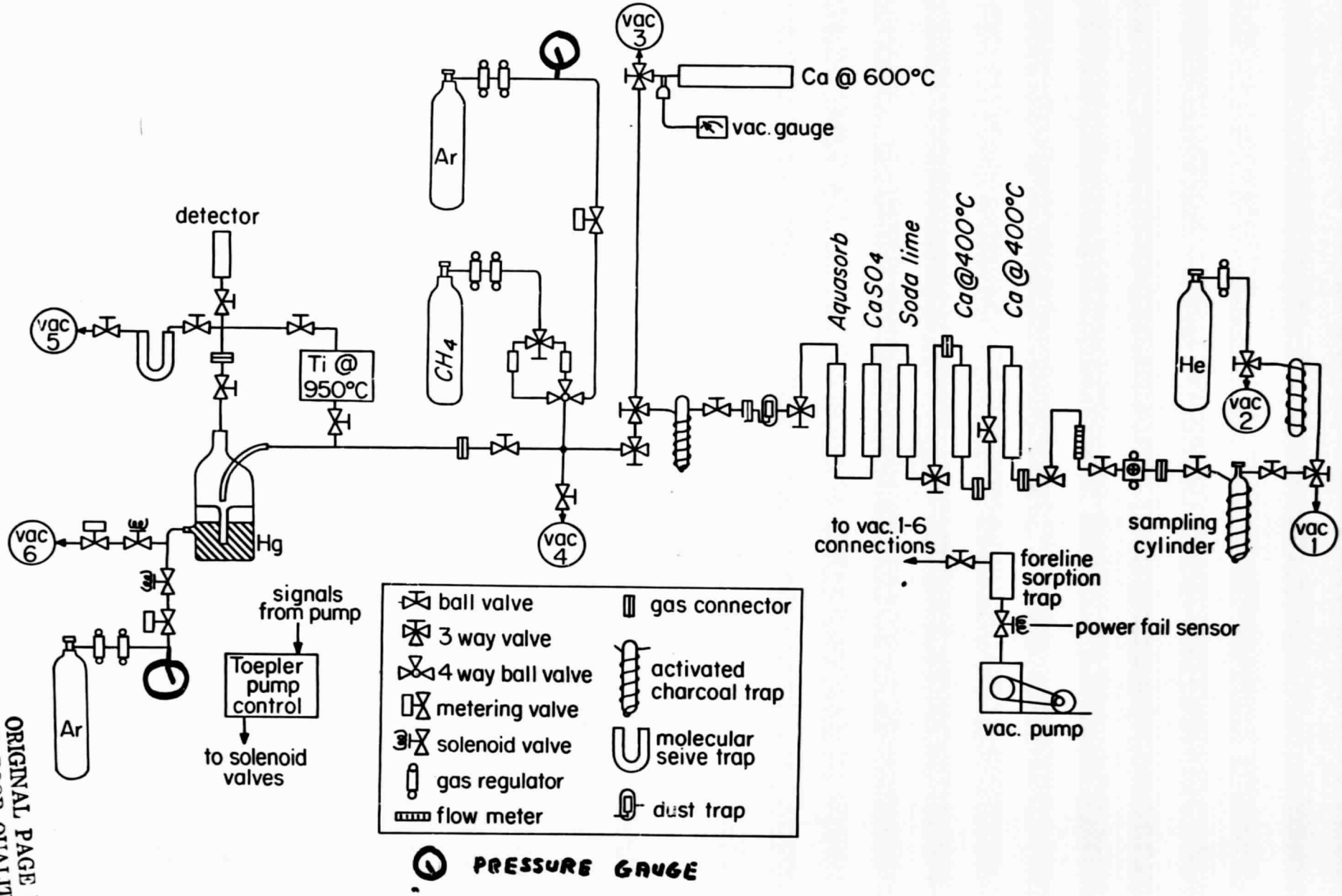


Figure 10

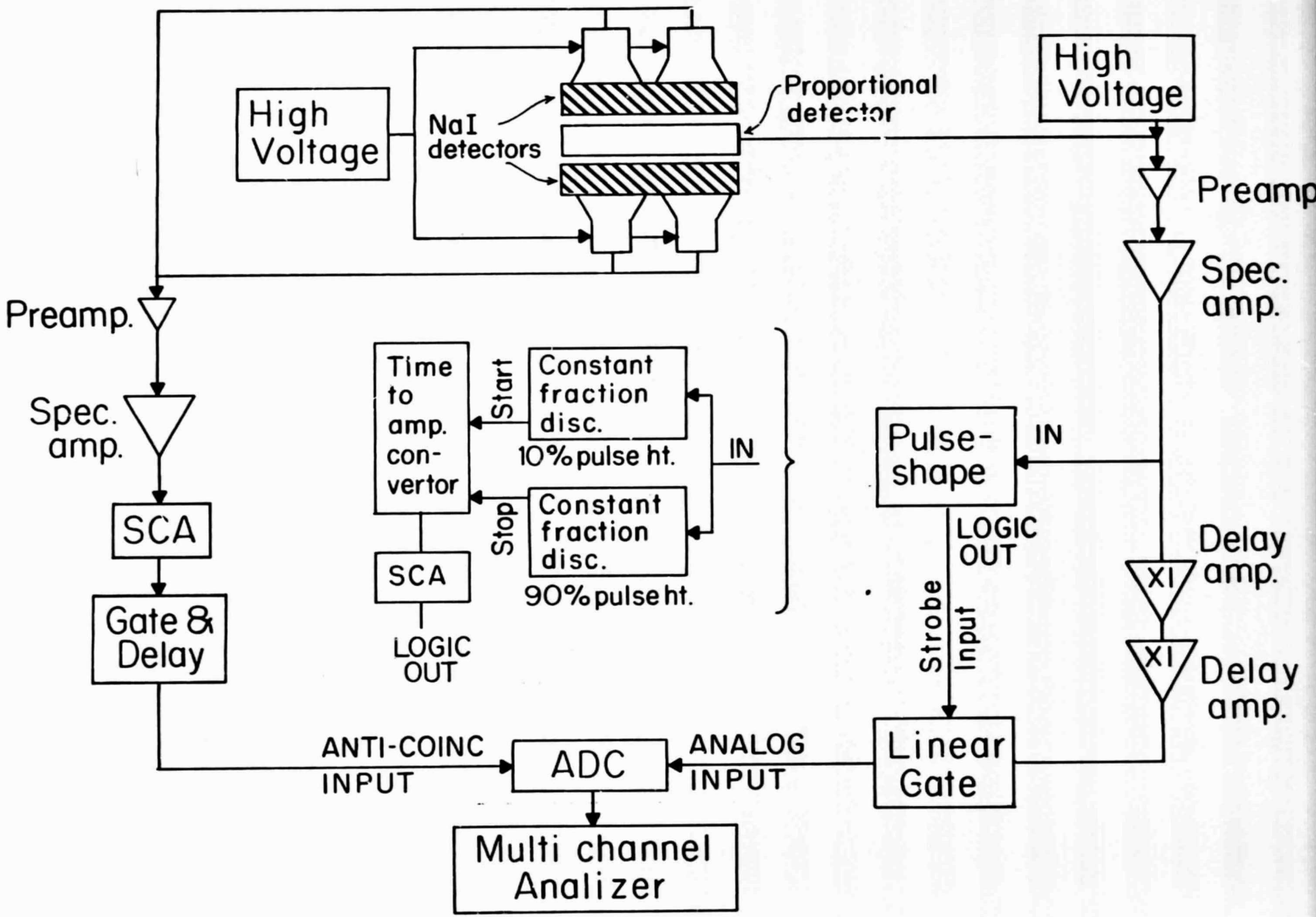

High Voltage
NaI detectors
Proportional detector
High Voltage
Preamp
Spec. amp.
Preamp.
Spec. amp.
Time to amp. con-vertor
Start
Constant fraction disc.
10% pulse ht.
IN
Stop
Constant fraction disc.
90% pulse ht.
SCA
LOGIC OUT
Pulse-shape
IN
LOGIC OUT
Strobe Input
Delay amp.
XI
XI
Delay amp.
SCA
Gate & Delay
ANTI-COINC INPUT
ADC
ANALOG INPUT
Linear Gate
Multi channel Analizer

Figure 11

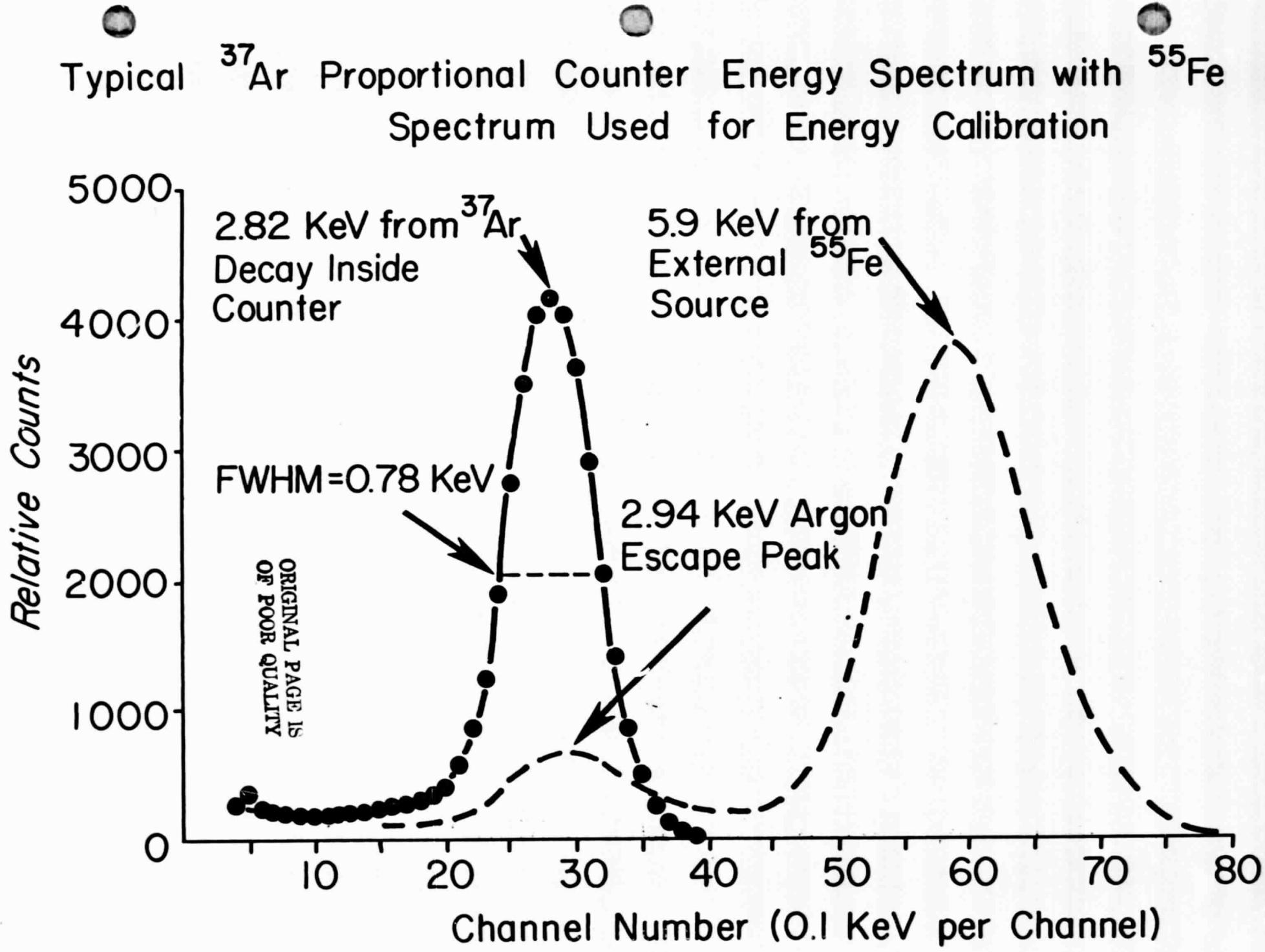

Typical ^{37}Ar Proportional Counter Energy Spectrum with ^{55}Fe Spectrum Used for Energy Calibration
2.82 KeV from ^{37}Ar Decay Inside Counter
5.9 KeV from External ^{55}Fe Source
FWHM=0.78 KeV
2.94 KeV Argon Escape Peak
ORIGINAL PAGE IS OF POOR QUALITY
Relative Counts
5000
4000
3000
2000
1000
0
10
20
30
40
50
60
70
80
Channel Number (0.1 KeV per Channel)

Figure 12

PROPORTIONAL DETECTORS

Type I - Wall material stainless steel, brass or copper depending on application

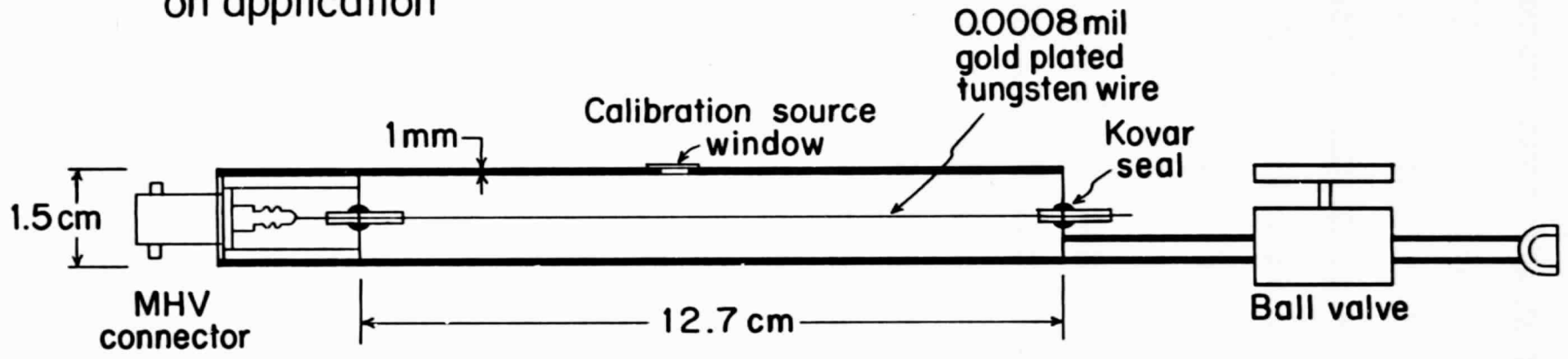
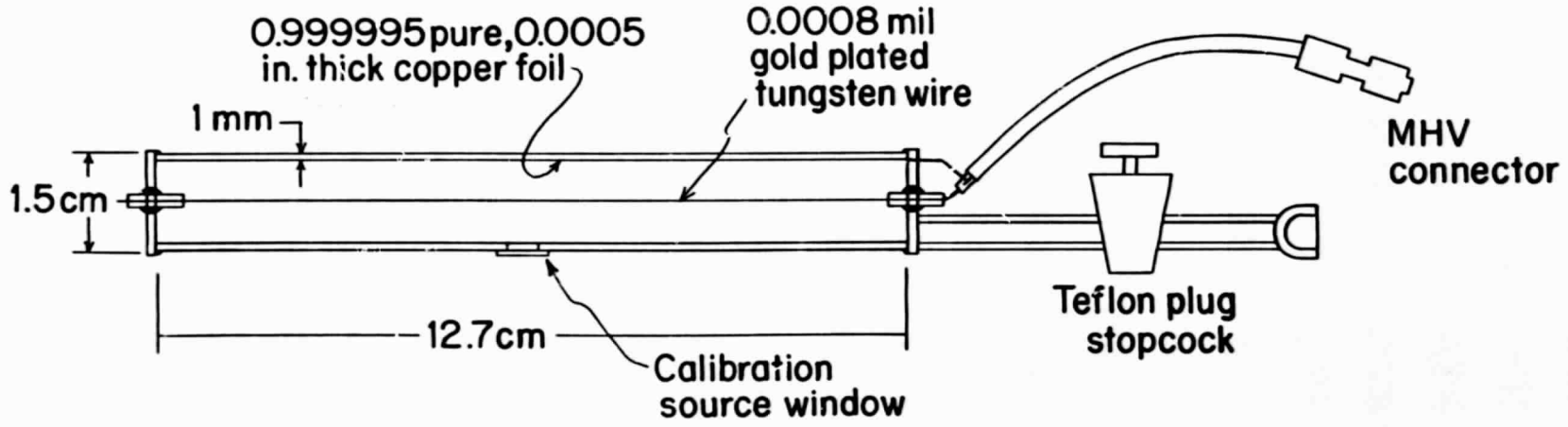


Figure 13

Type II - Wall material zone refined quartz



ends and a 0.0008 inch diameter gold-plated tungsten wire which is secured to the external portion of the seals with a silver-tin solder. A standard MHV connector is soldered to an extension on the copper tube and is also soldered to one of the Kovar seals. In addition, a 0.001 inch thick tin foil is soldered over a 3 mm hole in the center of the tube to allow the detector to "see" the ^{55}Fe calibration source. The energy resolution of ^{55}Fe is typically 17% and the background counting rate under the ^{37}Ar peak is normally 0.1 cpm.

The second type of detector is used for multiple day counting. The basic design is identical to the Type I detectors except that the tube material is zone refined quartz glass and ultra-pure 0.0005 inch thick copper foil and is used to line the inside of the tube. The energy spectrum is the same as the first type of detector, but the background rate is normally 0.03 cpm.

5.2 Discussion

The system has proven to be quite reliable with the only major problem encountered being the slow build up of tritium contamination inside the detectors. The tritium, which is present in the Health Sciences environment, causes a slow increase in background counting rates. The problem is controlled through cyrogenic trapping in the purification system and periodic pumping of the detectors with a titanium furnace (at 905°C) (7).

Potential applications of the ^{37}Ar systems to regional bone mass studies on lowering the TBC dose to 1 mrad will require counting samples for 3 to 5 days. The counting system could be expanded to handle seven detectors continuously, thus allowing a sample to be processed and placed in a detector for counting at the rate of one per day. Design studies for such a system have been performed and discussed in recent reports (13).

6.0 Excretion Rate

6.1 Measurement Technique

Sixteen patients who were undergoing serial examinations of body calcium by the ^{49}Ca technique at the cyclotron facility were studied during the early period of this research contract. The neutron beam produced at the cyclotron facility has a mean energy of 8 MeV and a full width at half maximum of 8 MeV and in these procedures, both the $^{48}Ca(n,\alpha)\ ^{49}Ca$ and the $^{40}Ca(n,\alpha)\ ^{37}Ar$ reactions are produced in-vivo.

Argon-37 excretion was measured by taking several samples of the exhaled breath at various times after irradiation. Continuous breath collections were not possible because the breath collection apparatus could not be used during the three, 12 minute total-body counting procedures after irradiation (for ^{49}Ca). The ^{37}Ar was processed and counted in early versions of the system previously described in this report. The breath samples were typically 10 minutes long.

One additional volunteer was studied for excretion rate at the 14 MeV facility when it was operational. The procedures were essentially the same as used at the cyclotron.

6.3 Excretion Rate Data

The combined data is shown on Figure 14. The vertical axis indicates the ^{37}Ar excretion as a rate normalized to the TBC of each patient as measured independently by the ^{49}Ca system. The data indicate the maximum rate of ^{37}Ar excretion occurs essentially immediately. By six hours post irradiation, the rate has decreased to approximately 1% of the maximum value.

Figure 15 depicts the serial excretion rates from three individuals and is typical of that seen in patients when three or more serial samples are obtained. The data of Figure 15 represent the two extremes observed in the combined data plot of Figure 14.

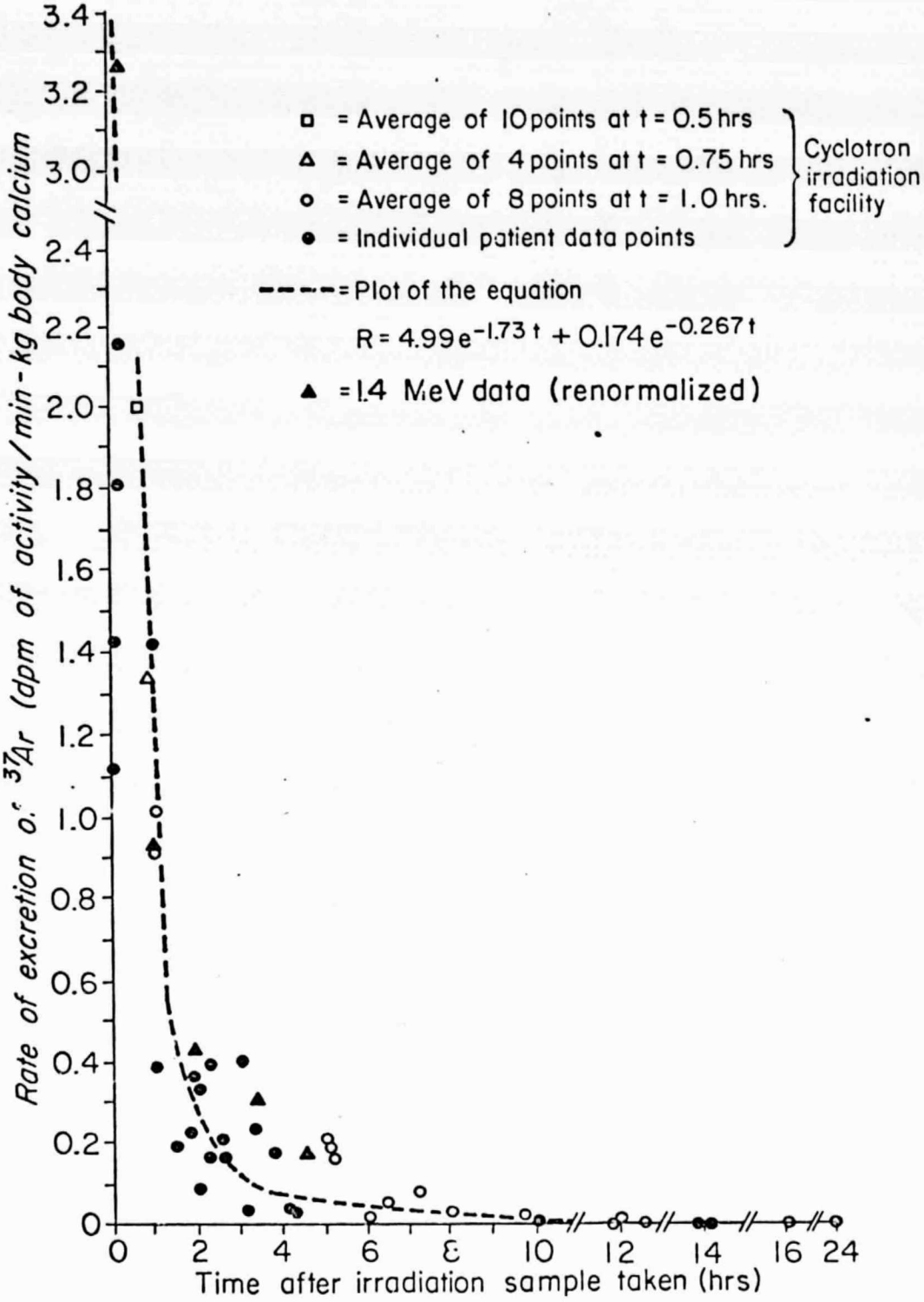


Figure 14 – Argon excretion data

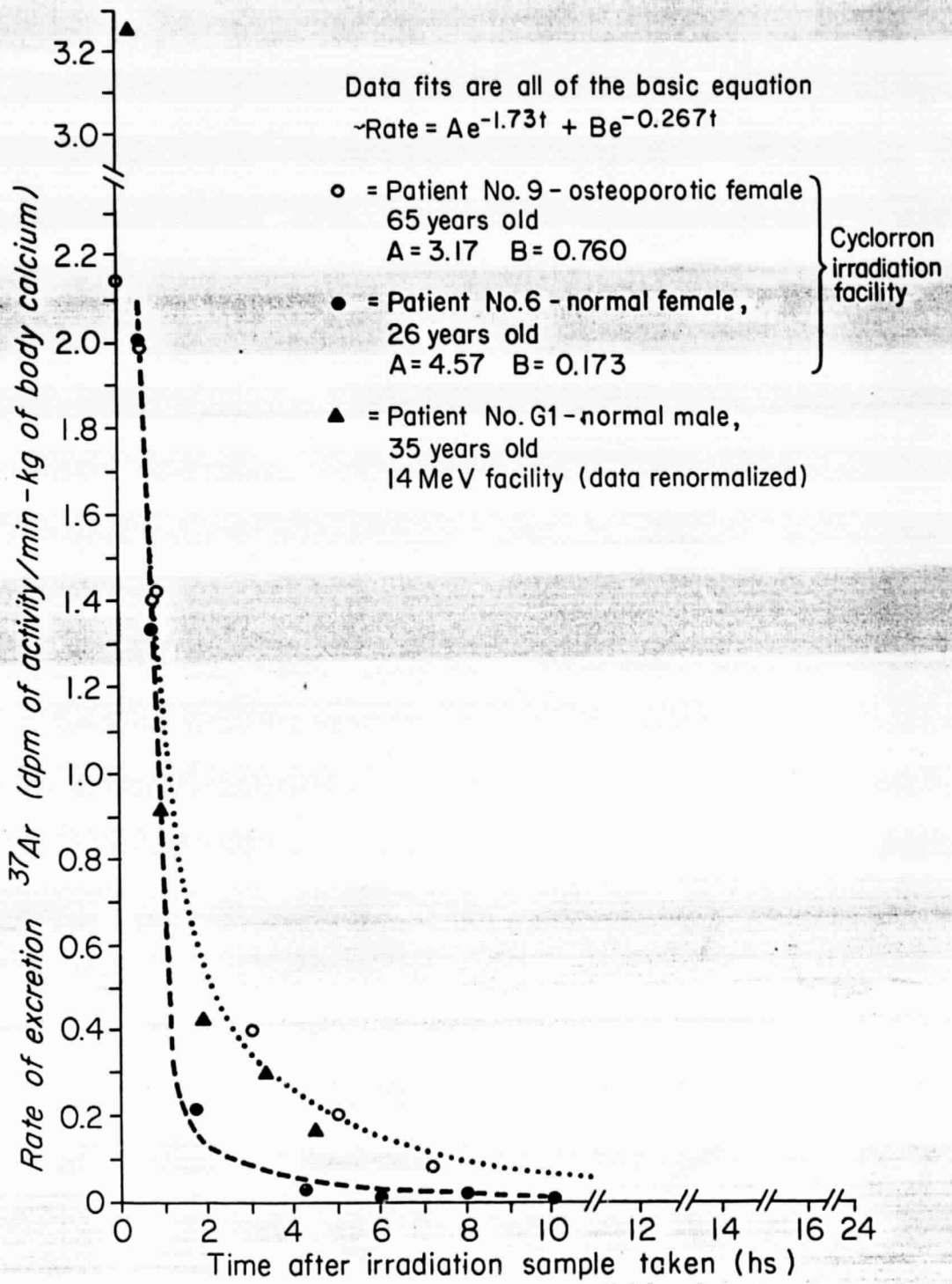


Figure 15 – Examples of argon excretion rates for individual patients

6.3 Discussion

The combined data can be described by a double exponential equation as indicated in Figure 14. The fast component of the descending limb has a T-1/2 of approximately 27 min. and the T-1/2 of the slow component is approximately 156 min. The individual data (Fig. 15) can be "fit" holding the T-1/2 values constant and adjusting the multiplicative constants of each exponential. Earlier analyses of this data (12) suggested that the fast T-1/2 values might be related to the surface area of bone, i.e. primarily turbecula bone. However, subsequent analyses has proven that such cannot be the case since models based on the excretion rate data and ^{37}Ar yields measured during studies to determine the accuracy of the technique show that 81% of the ^{37}Ar released is in the fast component and 18% is in the slow component (13). Since 90% of the surface area of bone is contained in turbecula bone which in turn comprises about 20% of total bone, the fast component could only contain 26% of the ^{37}Ar released if the surface area theory was correct.

7.0 Accuracy of Measurement

7.1 Measurement Technique

A study designed to determine the accuracy of the ^{37}Ar technique in measuring total body calcium (TBC) in humans was initiated during this reporting period. The study is based on measuring the TBC of a patient with the established ^{49}Ca system at the University of Washington and comparing the TBC to the ^{37}Ar yield from the same patient after irradiation at the 14 MeV facility. The original protocol required that the ^{37}Ar exhaled during the first hour, the first three hours, and the first five hours post irradiation be quantitated and compared to the ^{49}Ca TBC. However, after the first few irradiations, it

became clear that the 0-5 hour data will not be necessary since adequate accuracy can be obtained with the shorter breath collection intervals. As a result, the 0-5 hour collection period was generally not taken.

7.2 Accuracy Data

The study includes 10 normal volunteers and 4 osteoporotic patients (with documented compression fractures). The average age of the normal group is 57 years, with only one volunteer less than 30 years old. The group includes 6 males and 4 females. The volunteers ranged in height from 155 cm to 183 cm; in weight from 58.6 kg to 202.5 kg; and in TBC from 594 g to 1,137 g.

The data obtained to-date is listed in Table 1 and illustrated in Figure 16. The data shows a strong correlation between ^{37}Ar yield and TBC as measured using the ^{49}Ca technique.

7.3 Discussion

The correlation between the ^{37}Ar yields and TBC as measured by the ^{49}Ca technique is excellent ($r = 0.96$ for a one hour breath collection period, $r = 0.98$ for a three hour breath collection period). The least squares linear regression analysis describes the data well (the solid lines in Figure 16) and provides a standard area of the estimate (the dashed lines in Figure 16) for the regression which is within $\pm$ 5%. Thus, the TBC as measured by the ^{49}Ca technique can be predicted using the ^{37}Ar data to within $\pm$ 5% (1 S.E.). The predictability by the three hour breath collection data is slightly better than the one hour data, as clearly shown by the data point spread in Figure 16. For a one hour breath collection, the conversion of ^{37}Ar yield to TBC is:

$$\text{TBC (kg)} = 0.11 \left[{}^{37}\text{Ar one hour yield in pCi}\right] + 0.4.$$

For a three hour breath collection, the appropriate relation is:

$$\text{TBC (kg)} = 0.086 \left[{}^{37}\text{Ar three hour yields in pCi}\right] + 0.32.$$

Table I

Patient Data - ^{37}Ar Accuracy Study

Patient Number	Patient Sex	Patient Weight (kg)	Patient Height (cm)	Normal or Osteo.	Patient age (years)	^{44}Ca TBC (grams)	^{37}Ar Yield 0 - 1 hour pCi	^{37}Ar Yield 0 - 1 hour pCi/kg	^{37}Ar Yield 0 - 3 hour pCi	^{37}Ar Yield 0 - 3 hour pCi/kg	^{37}Ar Yield 0 - 5 hour pCi	^{37}Ar Yield 0 - 5 hour pCi/
G-1	M	76.8	183	N	45	1099	8.2	7.4	10.5	9.6	12.2	11.
G-2	F	77.3	163	N	26	820	5.8	7.1	7.8	9.5	9.0	11.
G-3	M	88.7	171	N	49	1138	7.8	6.9	10.4	9.1	12.1	10.
G-5	F	60.9	155	N	79	594	4.0	6.9	5.0	8.4	NA	NA
G-6	M	101.5	177	N	75	1005	NA	NA	8.8	8.8	NA	NA
G-7	M	81.6	178	N	66	1147	7.2	6.3	9.5	8.3	NA	NA
G-8	F	58.6	168	N	60	767	5.4	7.1	6.7	8.8	7.1	9.
G-9	M	80.5	171	N	67	1167	7.7	6.6	9.9	8.5	NA	NA
G-11	M	79.9	175	N	55	1116	8.4	7.5	10.4	9.3	11.4	10.
G-12	F	55.5	161	N	59	712	4.6	6.4	5.7	8.0	NA	NA
G-13	M	61.8	162	O	85	677.7	2.8	4.2	4.8	7.1	NA	NA
G-14	F	62.5	162	O	55	969.3	5.7	5.9	9.0	9.3	NA	NA
G-16	F	54.5	157	O	66	586.3	4.1	7.0	5.3	9.0	NA	NA
G-17	M	93.4	189	O	44	1129.9	7.7	6.8	9.6	8.5	NA	NA
Excluding G-13						Mean		6.8		8.0		10.
						σ		$\pm$ .46		$\pm$ 0.5		$\pm$ 0.
						% σ		$\pm$ 6.8%		$\pm$ 5.6%		$\pm$ 7.

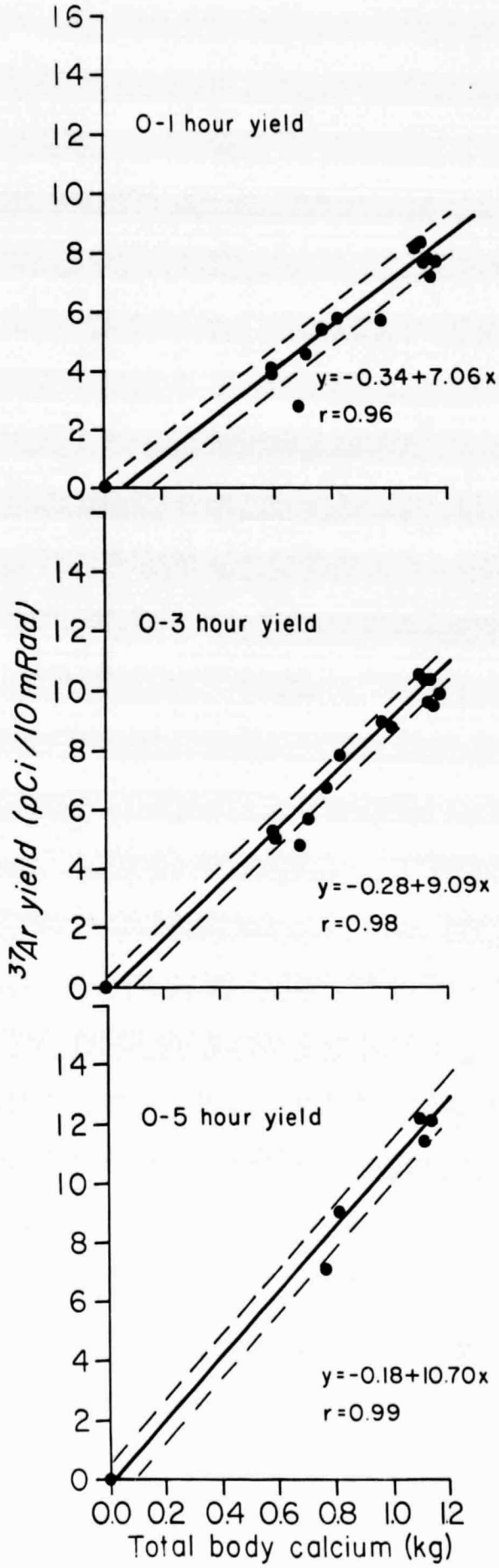

0-1 hour yield
y=-0.34+7.06x
r=0.96
0-3 hour yield
y=-0.28+9.09x
r=0.98
0-5 hour yield
y=-0.18+10.70x
r=0.99
37Ar yield (pCi/10 mRad)
Total body calcium (kg)
0.0 0.2 0.4 0.6 0.8 1.0 1.2

Figure 16

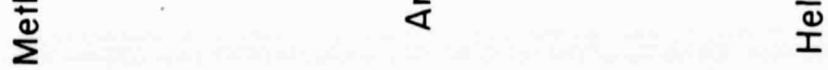


Figure 9

8.0 Precision of Measurement

8.1 Measurement Technique

A study to determine the precision of measurement of the ^{37}Ar TBC system was undertaken. Six normal volunteers were irradiated, each three times over approximately two weeks. The 0-1 and 0-3 hour ^{37}Ar yields were measured using the standard TBC procedure.

8.2 Precision Data

The ^{37}Ar yields were determined for each of the measurements of each of the six volunteers. In order to combine the results from all six individuals, the mean yield for each of the three measurements for each individual was calculated. The yields for each individual were then expressed as the ^{37}Ar produced relative to the mean yield for that individual.

The volunteers included 4 females and 2 males, ranging in age from 22 to 67 years old. The data is summarized in Table 2.

8.3 Discussion

The precision of the technique is quite acceptable at $\pm$ 3.3% (1 S.D.) for a breath collection period of one hour and $\pm$ 2.4% for a three hour breath collection. The slightly better precision for the three hour breath collection is most likely due to a combination of slightly better counting statistics and less biological variation due to slight changes in the excretion rate (see Section 6.2).

9.0 Summary and Discussion

9.1 General

The systems have proven to be quite reliable with the only major maintenance worry being the prevention of leaks, primarily in the rebreathing

TABLE 2

^{37}Ar Yield after Repetitive Irradiations

Neutron Source = 14 MeV Facility

Patient Dose = 10 mrad/Study

Patient Study No.	Sex	Age	Irradiation Date	Breath Collection Interval (Hours Post Irradiation) 0 - 1 ^{37}Ar Yield (picocuries)	0 - 1 Relative Yield*	0 - 3 ^{37}Ar Yield (picocuries)	0 - 3 Relative Yield*
G - 4A	F	49	2-14-76	3.85	0.967	5.44	0.983
G - 4B			2-16-76	4.07	1.022	5.63	1.017
G - 4C			3-02-76	4.03	1.011	Lost	
G - 10A	M	67	5-11-76	6.76	0.985	9.86	0.979
G - 10B			5-13-76	6.48	1.016	10.16	1.009
G - 10C			5-25-76	6.86	0.999	10.20	1.013
G - 15A	M	57	10-19-76	7.46	1.014	13.17	1.021
G - 15B			11-02-76	7.56	1.028	13.07	1.013
G - 15C			11-09-76	7.05	0.958	12.47	0.966
G - 18A	F	24	4-20-77	3.09	1.002	4.88	1.000
G - 18B			5-06-77	3.18	1.031	5.01	1.027
G - 18C			5-27-77	2.98	0.966	4.75	0.973
G - 19A			4-25-77	1.25	0.997	2.16	0.997
G - 19B	F	22	5-02-77	1.20	0.963	2.11	0.974
G - 19C			6-15-77	1.29	1.035	2.23	1.029
G - 20A			4-22-77	3.32	0.933	4.50	0.953
G - 20B	F	30	5-04-77	3.57	1.004	4.79	1.015
G - 20C			5-18-77	3.78	1.063	4.87	1.032
Combined S.D.					± 3.3%		± 2.4%

system due to normal wear of flexible joints and hoses. The question of potential health risks due to patient dose of 10 mrad has also been evaluated and found to be negligible (14).

The excellent correlation between the ^{37}Ar yield and TBC as measured by the ^{49}Ca system ($r = 0.98$, standard error of the estimate $\simeq \pm 5\%$) coupled with the patient dose of 10 mrad meet the primary objective of this contract to develop a low dose technique to accurately and precisely measure total body calcium.

9.2 Factors Affecting Accuracy and Precision

In addition to the usual problems of biological variance and the quality control of the collection and purification systems to prevent gas leaks, there are three major areas of concern in utilizing the ^{37}Ar technique: 1) long-term trapping of ^{37}Ar in bone crystal; 2) the incomplete collection of the ^{37}Ar released and the cause of the excretion rate half-lives; and 3) the possibility of reabsorption of ^{37}Ar from rebreathing system back into fatty tissues within the patient.

There have been several indications that some of the ^{37}Ar produced in-vivo is retained (5,7,9,12,13). Estimates made by our group, H.E. Palmer, and others, which are based upon the amount of ^{37}Ar obtained per gram of calcium from irradiated solutions of calcium as compared to the amount of ^{37}Ar recovered from in-vivo irradiations of human subjects, indicate that 10 to 30% of the argon may be retained.

The accuracy data presented in Section 7 indicates that whatever retention is present is having no discernible effect on the application of the ^{37}Ar technique. Some data recently obtained at Sloan-Kettering Cancer Center gives some indication of why retention is not a major problem. The data is an attempt

at measuring percent Ar retention vs. age in bone removed from amputated limbs. The experimental procedure involves sealing the "fresh" bone in a glass container filled partially with water. The sample is irradiated and helium bubbled through the container. The ^{37}Ar then recovered is considered as the normally released fraction. The bone is then dissolved and heated in solution while helium is bubbled through. The ^{37}Ar recovered is considered as the retained fraction. The normally released fraction, since it is still determined via a totally in-vitro procedure, does not completely duplicate the in-vivo process. However, the character of the change of percent ^{37}Ar retained vs. age probably is representative of the in-vivo process.

The data is presented in Figure 17. A least squares fit to the data is also plotted in the figure. An anlyses of this data (13) indicate that worse possible "error" or change to be expected over a 50 year span if corrections are not made for retention is 4%. The conclusion is further verified by plotting the ^{37}Ar yields obtained in the accuracy study and correcting for retention using the Bigler data. Such a plot is shown on Figure 18. While the slope has changed, the standard error of the estimate is slightly worse, and hence, corrections for long-term retention of ^{37}Ar are not necessary.

The second major concern, the incomplete collecting of all the ^{37}Ar which is released in-vivo, is only a problem if the excretion at any point in time does not represent a uniform sampling of the body calcium, or if there are extreme variances in the excretion rate curves between individuals. The questions of the cause for the biological half-lives observed on the excretion rate data remains unanswered, except that it does not arise from simple trabecular and cortical bone compartments (Section 6). In view of the excellent accuracy data (Section 7), it appears that the observed ^{37}Ar components are sampling the total body calcium rather uniformly.

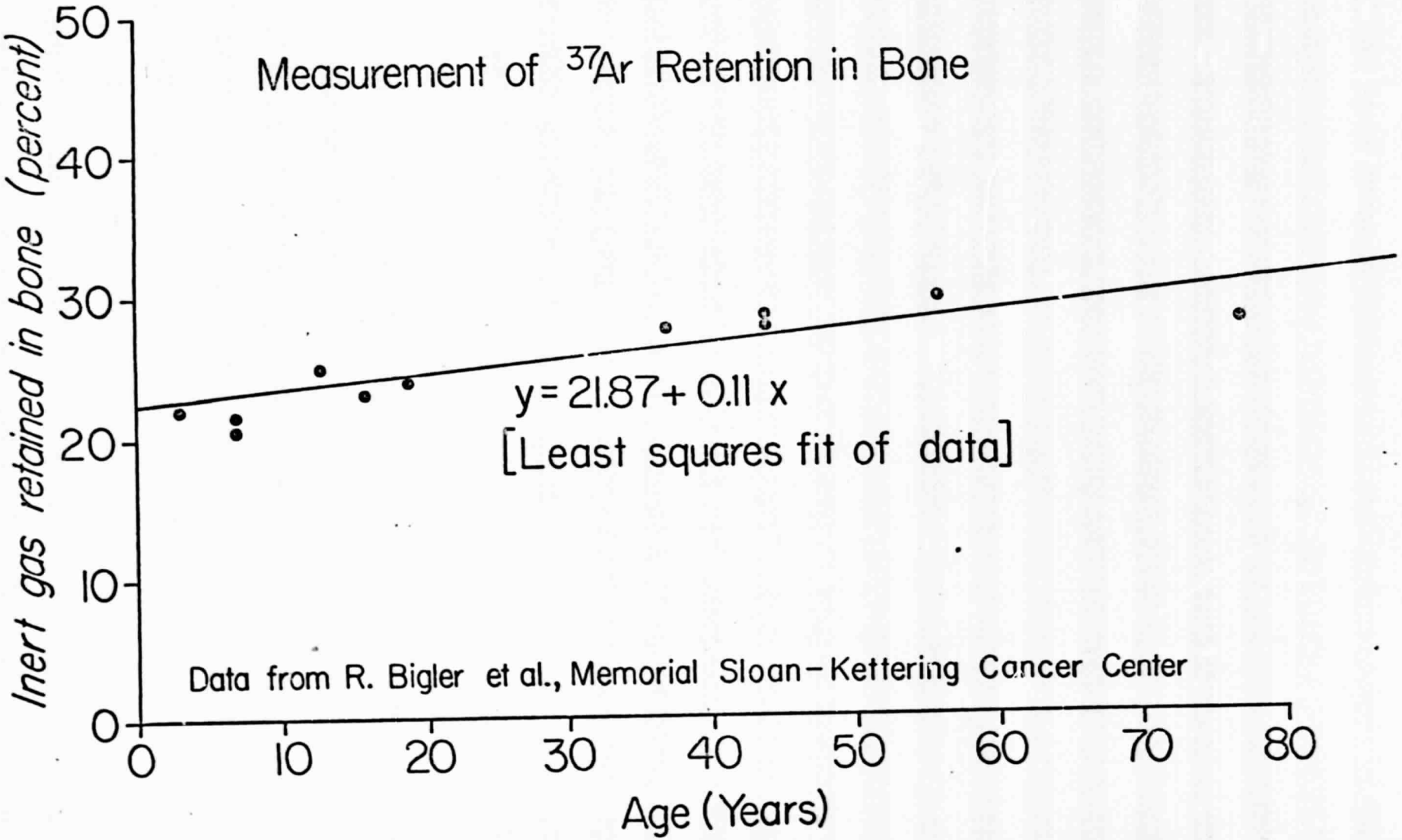

Measurement of ^{37}Ar Retention in Bone
y = 21.87 + 0.11 x
[Least squares fit of data]
Data from R. Bigler et al., Memorial Sloan-Kettering Cancer Center
Inert gas retained in bone (percent)
Age (Years)
0
10
20
30
40
50
60
70
80

FIGURE 17

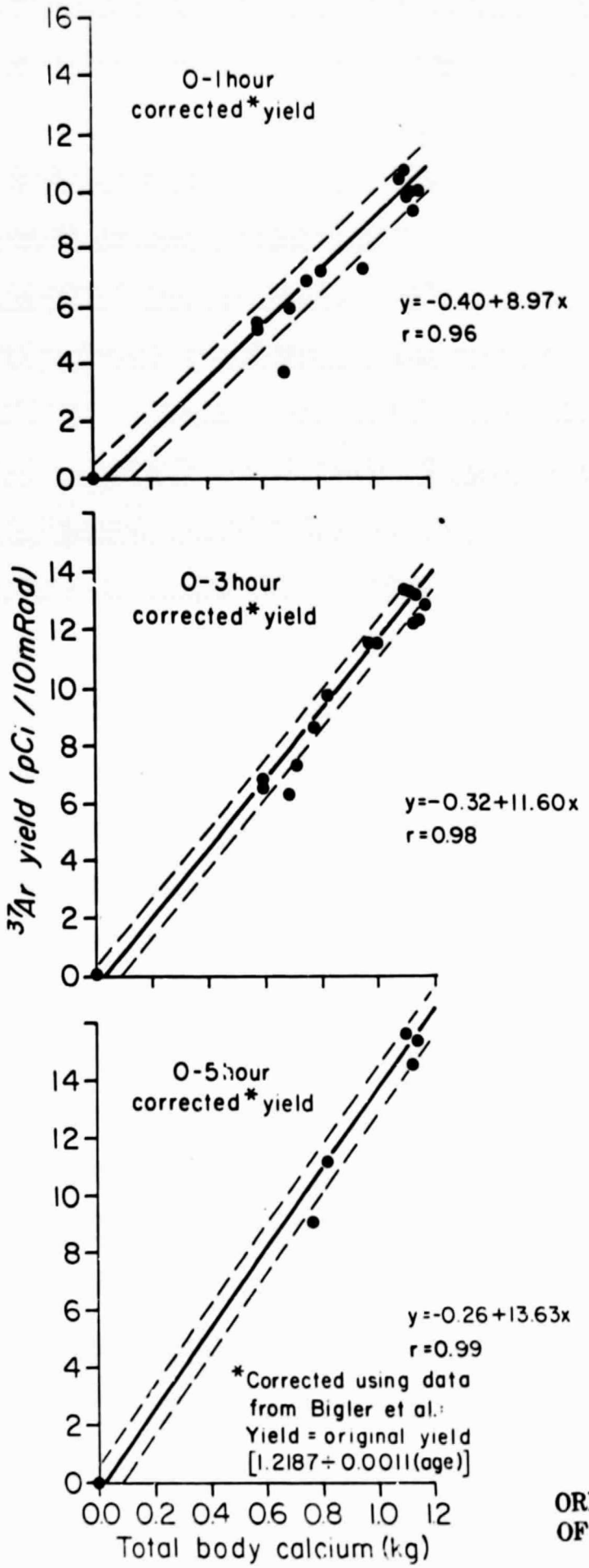


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Figure 18

The only major concern regarding the use of ^{37}Ar is the possibility of reabsorption of ^{37}Ar into the body from the circulating gases of the rebreathing system. This question has already been discussed in detail in Section 3.2, and based on the accuracy and precision data does not appear to be a major factor.

Further support for this conclusion can be obtained by correlating ^{37}Ar yields versus weight and body size. Analysis of this type yield no correlation. Since reabsorption would presumably be more severe in "stout" individuals with more body fat, the lack of correlation would indicate that any reabsorption has no observable effect.

However, a direct investigation of the reabsorption question is planned by direct measurement of the reabsorption of ^{41}Ar when introduced into the circulating atmosphere of the rebreathing system. Results of such measurements are expected to be published during 1978.

9.3 Recommendations

The results of the accuracy and precision studies clearly indicate that the ^{37}Ar technique can be used to measure total body calcium in humans. The implementation of the system for large scale clinical studies or for research into bone metabolism would benefit from development of an expanded purification and counting system. The existing system has been assembled primarily as an engineering developmental system, and not as a high use system. During the past year and a half, no major changes in the design of the system have been needed to analyze the patient data, although several revisions to decrease system down time would be desirable.

We have designed a new purification system. The basic system design will be the same as the existing apparatus, but will include two complete purification

systems with cross connections at each major component. Thus, if all components are operational, two samples at a time can be processed. If part of one system needs repair, two samples could still be processed by appropriate sharing of system resources.

The new system would include provisions for the addition of automatic valve activators, as well as pressure, flow and temperature sensors which would allow the system to be run by a computer. Increasing throughput to four or six samples a day could be accomplished, if needed.

Other improvements in the new system would include: 1) no tygon or other "weak" vacuum materials will be used, 2) reduction of the copper columns will be done automatically without removing the columns from the system, 3) the addition of connection ports for the helium leak detector for preventive maintenance, 4) replacement of all brass valves and tubing with stainless steel to prevent mercury-induced material failures, 5) automatic liquid nitrogen level controllers for all dewars, and 6) a vacuum system providing higher pumping speeds.

If a new purification system is built, the counting system will also need to be expanded to handle the larger number of samples to be counted per day. The multichannel analyzer should be provided with a gated router to allow the collection of multiple spectra. Several more proportional detectors should be fabricated and a new cosmic-ray anti-coincidence shield should be fabricated to allow proper shielding of multiple proportional detectors. With a relatively modest expansion of the existing system, up to eight detectors could be operated simultaneously. Typically, one of the detectors would be a background monitor, while the remainder of the detectors would be available for sample counting.

Support of research at sites other than the University of Washington would be most efficiently implemented by installing activation facilities at the selected sites (or develop a mobile activation facility on a trailer) and sending the breath samples to the central processing and counting facility at the University of Washington.

10.0 References

1. Nelp WB, Palmer HE, Murano R, et al: Measurement of total body calcium (bone mass) in-vivo with the use of total body neutron analysis. J Lab Clin Med 76: 151-162, 1970
2. Nelp WB, Denney JD, Murano R, et al: Absolute measurements of total body calcium (bone mass) in-vivo. J Lab Clin Med 79: 430-438, 1972
3. Cohn SH, Dombrowski CS: Measurement of total body calcium, sodium chlorine, nitrogen and phosphorus in man by in-vivo neutron activation analysis. J Nucl Med 12: 499-505, 1971
4. Chamberlain MJ, Fremlin JH, Peters DK, et al: Total body calcium by whole body neutron activation: New technique for study of bone disease. Br Med J 2: 581-585, 1968
5. Lewellen TK, Nelp WB: Annual report to NASA on work completed on total body calcium analysis under contract NASA-13029, November 1, 1972 to October 1, 1974. November 1974
6. Palmer HE: Feasibility of determining total body calcium in animals and humans by measuring ^{37}Ar in expired air after neutron irradiation. J Nucl Med 14: 522-527, 1973
7. Lewellen TK, Nelp WB: Annual report to NASA on work completed on total body calcium analysis under contract NASA-13029, October 1, 1973 to October 1, 1975. October 1975
8. Nelp WB, Denney JD, Murano R: Absolute measurement of total body calcium (bone mass) in-vivo. J Lab Clin Med 79:430-438, 1972
9. Bigler RE, Laughlin JS, Davis R, Evans JC: Quantitative analyses of the fate in man of ^{37}Ar produced in body calcium by fast neutron exposure. Rad Res 67: 266-276, 1976

10. Malkin VB: Foundations of Space Biology and Medicine, NASA Vol. II, pg 17, 1975

11. Behuke AD, Thomason RM, Shaw LA: The rate of elimination of dissolved nitrogen in man in relation to the fat and water content of the body. Amer J Physiol 114: 137-146, 1936

12. Lewellen TK, Chesnut CH, Nelp WB: Preliminary observations of excretion of ^{37}Ar from man following whole body neutron activation - an indicator of total body calcium. J Nucl Med 16:672-675, 1975

13. Lewellen TK, Nelp WB: Annual report to NASA on work completed on total body calcium analysis under contract NASA-13029. October 1, 1975 to October 1, 1976. October 1976

14. Lewellen TK: Special report to NASA - Health risks due to neutron dose in ^{37}Ar TBC measurements - an evaluation. August 1976

11.0 Papers Presented

1.	Measurements of Bone Mass	Nucl. Med. Post-Grad Seminar Colby College Maine	8/74	Dr. Nelp
2.	The Validity of TBC by NAA	FDA - Metabolic Advisory Committee	2/75	Dr. Nelp
3.	Dianabol and Osteoporosis	FDA - " " "	2/75	Dr. Chesnut Dr. Baylink
4.	Body Composition Studies Measuring Total Body Activation Products in Exhaled Breath	3rd National Conference on Small Accelerators - North Texas State Univ. Denton, Texas	10/74	Mr. Palmer
5.	Body Composition Studies Measuring Total Body Activation Products in Exhaled Breath	Sloan-Kettering Cancer Institute New York City	12/74	Mr. Palmer
6.	Body Composition Studies Measuring Total Body Activation Products in Exhaled Breath	Brookhaven National Laboratories New York	12/74	Mr. Palmer
7.	Efficacy of Dianabol in Osteoporosis as Determined by Changes in Total Bone Mineral Mass	2nd Annual Affiliate Conf. of the Center for Research in Oral Biology Seattle, WA	5/74	Dr. Chesnut
8.	Quantitation of Bone Mass in Osteoporosis - Recent Advances	XIth European Symposium on Calcified Tissues - Copenhagen, Denmark	5/75	Dr. Chesnut
9.	^{37}Ar Excretion as an Indication of Total Body Calcium	Sloan-Kettering Cancer Institute	6/75	Dr. Lewellen
10.	Evaluation of Kinetics of ^{37}Ar Excretion from Man	2nd E. Kilbride Conf. on Prog. & Prob. of in-vivo Act. Anal. Glasgow, Scotland	4/76	Dr. Nelp
11.	Gaseous Radionuclides Excreted in Expired Air after Neutron Irradiation and their relation to Body Composition	"	4/76	Mr. Palmer

12.	TBIVNAA for TBC $^{40}Ca(n,\alpha)^{37}Ar$ Breath collection, gas purification and analysis	2nd E. Kilbride COnf. on Prog. & Prob. of in-vivo Act. Anal. Glasgow, Scotland	4/76	Dr. Lewellen
13.	14 MeV TBNAA $^{40}Ca(n,\alpha)^{37}Ar$ The patient irradiation system-uniformity and dosimetry	"	4/76	Dr. Lewellen
14.	Nuclear medicine techniques in the evaluation of post-menopausal osteoporosis	SNM National Convention Dallas, Texas	6/76	Dr. Chesnut
15.	Measurement of total body calcium by ^{37}Ar Excretion	"	6/76	Dr. Lewellen
16.	Measurement of total body calcium utilizing the $^{40}Ca(n,\alpha)^{37}Ar$ reaction	NASA Radiation Safety Committee, JSC Houston, Texas	8/76	Dr. Lewellen

12.0 Papers Published

1. Chesnut CH, Manske E, Baylink D, et al: Preliminary Report - correlation of total body calcium (bone mass), as determined by photon absorption. Int. Conf. on Bone Mineral Measurement, pg 34, Chicago 1973

2. Lewellen TK, Nelp WB, Palmer, et al: Argon-37 excretion in humans following total body neutron activation. J Nucl Med 15:511, 1974

3. Lewellen TK, Nelp WB, Palmer HE: Argon-37 excretion in humans following total body neutron activation. Trans Amer Nucl Society 18:96, 1974

4. Chesnut CH, Nelp WB, Denney JD: Efficacy of Dianabol in osteoporosis as determined by changes in total bone mineral mass. J Nucl Med 15:483, 1974

5. Chesnut CH, Nelp WB, Baylink D: Efficacy of Dianabol in osteoporosis as determined by changes in total bone mineral mass. Clin Res 22:464, 1974

6. Palmer HE, Lewellen TK, Chesnut CH, et al: Body composition determination by measurement of total body activation products in expired air. Proc. 3rd Conf. on Application of Small Accelerators. AEC, October 1974

7. Manske E, Chesnut CH, Baylink, et al: Correlation of total body calcium (bone mass) and regional bone mass in osteoporosis. Metabolism 24:615, 1975

8. Chesnut CH, Nelp WB, Baylink, et al: Effect of Methandrostenolone on postmenopausal bone wasting as assessed by changes in total bone mineral mass. Metabolism 26:267-277, 1977

9. Lewellen TK, Chesnut CH, Nelp WB, et al: Preliminary observations on excretion of ^{37}Ar from man following whole body neutron activation - an indication of total body calcium. J Nucl Med 16:672, 1975

10. Chesnut CH, Nelp WB, Lewellen TK: Quantitation of bone mass in osteoporosis recent advances. Proceedings - XIth European Symposium in Calcified Tissues. Elsinore, Denmark. May 25-29, 1975. Calcified Tissues, Ed. S. Pors Nielsen and E. Jhørting-Hansen, FADL Publishing Co., Copenhagen, Denmark, p 370-374, 1976

11. Lewellen TK, Nelp WB, Murano R et al: Absolute measurement of total body calcium by the Argon-37 method - Preliminary results. J Nucl Med 18:929-932, 1977